Clinical cell tracking of mononuclear cells using magnetic resonance imaging and superparamagnetic particles of iron oxide


Centre of Cardiovascular Science
The University of Edinburgh
Cell Therapy for Cardiovascular Disease

- Post-myocardial infarction and heart failure
  - The BALANCE Study Yousef et al JACC 2009
  - The BOOST trial Wollert et al Lancet 2004
Magnetic Resonance Imaging

- High resolution
- No ionising radiation
- Serial imaging
Endorem

- Super-paramagnetic particles of Iron Oxide (SPIO)
- Particle-size 80-150nm
- Signal deficit on T2- and T2*-weighted imaging
Efficient magnetic cell labeling with protamine sulfate complexed to ferumoxides for cellular MRI


Recently, there have been several reports using various superparamagnetic iron oxide (SPIO) nanoparticles to label mammalian cells for monitoring their temporal and spatial migration in vivo by magnetic resonance imaging (MRI). The purpose of this study was to evaluate the efficiency and toxicity of labeling cells using 2 commercially available Food and Drug Administration (FDA)–approved agents, ferumoxides, a suspension of dextran-coated SPIO used as an MRI contrast agent, and protamine sulfate, conventionally used to reverse heparin anticoagulation but also used ex vivo as a cationic transfection agent. After labeling of human mesenchymal stem cells (MSCs) and hematopoietic (CD34+) stem cells and other mammalian cells with ferumoxides–protamine sulfate complexes (FE-Pro), cellular toxicity, functional capacity, and quantitative cellular iron incorporation were determined. FE-Pro–labeled cells demonstrated no short- or long-term toxicity, changes in differentiation capacity of the stem cells, or changes in phenotype when compared with unlabeled cells. Efficient labeling with FE-Pro was observed with iron content per cell varying between 2.01 ± 0.1 pg for CD34+ cells and 10.94 ± 1.86 pg for MSCs with 100% of cells labeled. Cell labeling using these agents should facilitate the translation of this method to clinical trials for evaluation of trafficking of infused or transplanted cells by MRI. (Blood. 2004;104:1217-1223)
Cell Labelling Protocol

**Veneesection/Apheresis**

**Isolation of PBMCs (Ficoll)**

**Labelling**
- Endorem
- Protamine sulphate
- Normal saline
- 2 hours, room temperature
Iron Uptake

Prussian Blue staining

~20 pg Fe/cell

10^5 cells
10^4 cells
10^3 cells
10^2 cells
Cell Viability

Annexin V binding/Propidium Iodide staining

N=4

Lactate dehydrogenase release

N=4
Release of inflammatory mediators

IL-1β

IL-10

IL-12p70

TNF
Arbab method

Edinburgh method
In vitro migration

Upper chamber: cell suspension (~250,000 cells) in RPMI

Microporous (5\(\mu\)m) membrane

Lower chamber: 600\(\mu\)l RPMI +/- mcp-1
<table>
<thead>
<tr>
<th>Chemokine</th>
<th>0ng/ml MCP-1</th>
<th>50ng/ml MCP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labelled (Arbab</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>method)</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>Labelled (Edinburgh method)</td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>Unlabelled</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
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</tbody>
</table>
In vitro migration

- Unlabelled cells
- Labelled cells

No of migrated cells

0 ng/ml MCP-1

50 ng/ml MCP-1
Clinical Study 1: Intramuscular Administration

Aims:

1. To investigate the safety of intramuscular administration of Endorem-labelled cells in healthy volunteers.
2. To demonstrate the potential to image labelled cells in vivo.
3. To establish the operational capacity and logistical issues that would be involved in conducting a patient study.
Intramuscular Administration

6 healthy volunteers

- Endorem Alone
- Labeled Cells 10^7 cells
- Unlabelled Cells 10^7 cells

MRI scanning
T2-weighted MRI of the thigh

- Endorem alone
- Labeled cells \((10^7)\)
- Unlabeled cells \((10^7)\)
Study 2: Intravenous Administration Phased-dosing protocol

Aim
To confirm the safety of intravenous administration of labelled cells
## Phased-dosing Protocol

<table>
<thead>
<tr>
<th>Visit Schedule</th>
<th>Cells obtained by</th>
<th>Dose/Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visit 1</td>
<td>Venesection</td>
<td>Dose 1 = $10^4$ cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dose 2 = $10^5$ cells</td>
</tr>
<tr>
<td>Visit 2</td>
<td>Venesection</td>
<td>Dose 3 = $10^6$ cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dose 4 = $10^7$ cells</td>
</tr>
<tr>
<td>Visit 3</td>
<td>Apheresis</td>
<td>Dose 5 = $10^8$ cells</td>
</tr>
<tr>
<td>Visit 4</td>
<td>Apheresis</td>
<td>Dose 6 = $10^9$ cells</td>
</tr>
</tbody>
</table>
Phased-dosing Protocol

- No early or delayed clinical side or adverse effects
- No haemodynamic effects
- No effect on clinical biochemistry
  - U&E, LFTs, Glucose
- No effect on clinical haematology including coagulation
  - FBC, PT, APTT, Fibrinogen, D-dimer, TAT complexes, thrombin fragments 1&2
Study 3
Intravenous Administration
MRI Scanning

Aim:
To demonstrate the potential to image labelled cells in vivo at a target site following intravenous administration.
Venesection (n=6) or leucapheresis (n=6)

Isolation of PBMCs (Ficoll)

Labelling
Endorem (100µg/ml)
Protamine sulphate (4µg/ml)
0.9% saline
room temperature, 2hrs

MRI scanning

Pre-infusion

1130

1300

1530

Post-infusion

Reinfusion

MRI scanning

0800

1130

1300

Pre-infusion

1530

Post-infusion
Siemens 3T Magnetom Verio (Siemens Medical Systems, Germany)

Body matrix coil and 2 spine matrix coil elements

T2*-weighted in-phase multi-echo, gradient-echo sequences (TE 4.1-22.1ms)

Liver and spleen
\[
T2^* = \frac{(TE_2 - TE_1)}{\ln(SI_1/SI_2)}
\]
T2* Map
Endorem-labelled Cells

Pre-cells

24hrs Post-cells

Liver

Spleen

Liver

Spleen

T2-weighted
T2* value following cell infusion

Venesection
~10⁸ cells
Liver p=0.01
Spleen p=0.001

Apheresis
~10⁹ cells
Liver p=0.001
Spleen p=0.001
In vivo migration

Noninvasive MR imaging of magnetically labeled stem cells to directly identify neovasculature in a glioma model

In vivo tracking of dual-labeled mesenchymal stem cells homing into the injured common carotid artery.

Homing of adipose-derived stem cells to radiofrequency catheter ablated canine atrium and differentiation into cardiomyocyte-like cells
Ung Kim a, Dong-Gu Shin b, Jong-Seon Park b, Young-Jo Kim b, Se-Il Park c, Young-Mi Moon c, Kyu-Shik Jeong d
Int J Cardiol 2009

MRI tracking of intravenously transplanted human neural stem cells in rat focal ischemia model
Miyeoun Song a,e, Youngju Kim a, Yoonha Kim a, Sun Ryu a, Inchan Song b, Seung U. Kim c,d, Byung-Woo Yoon a,e,* Neurosci Res 2009
Conclusions

1. SPIO-labelled mononuclear cells can be prepared for human cell-tracking studies under GMP-compliant conditions.

2. SPIO-labelling does not affect cell viability or function.

1. Intramuscular and intravenous administration of up to $10^9$ SPIO-labeled cells is safe.

2. SPIO-labeled cells can be imaged at a target site at clinically relevant fields strengths following intramuscular or intravenous administration.
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